(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 351 314 A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 89401997.5

(22) Date of filing: 11.07.89

(51) Int. Cl.[5]: A 61 L 33/00

(30) Priority: 11.07.88 JP 172360/88
11.07.88 JP 172361/88
09.08.88 JP 198555/88

(43) Date of publication of application: 17.01.90 Bulletin 90/03

(84) Designated Contracting States: BE DE FR GB IT NL SE

(71) Applicant: Terumo Kabushiki Kaisha
No. 44-1, Hatagaya 2-chome Shibuya-ku
Tokyo 151 (JP)

(72) Inventor: Hagiwara, Kazuhiko c/o Terumo K.K.
2656-1, Ohbuchi
Fuji-shi Shizuoka (JP)

Kitoh, Hitoshi c/o Terumo K.K.
2656-1, Ohbuchi
Fuji-shi Shizuoka (JP)

Oshibe, Yoshihiro
2-34, Rokkanyama Taketoyo-cho
Chita-gun Aichi (JP)

Ohmura, Hiroshi
5-3-1, Rokkanyama Taketoyo-cho
Chita-gun Aichi (JP)

(74) Representative: Gillard, Marie-Louise et al
Cabinet Beau de Loménie 55, Rue d'Amsterdam
F-75008 Paris (FR)

(54) Medical material and medical implement.

(57) A material in which functional groups are introduced on a substrate by such methods as coating or oxidation or through a compound containing hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) and in which heparin is covalently bonded to these functional groups either directly or through a coupling agent, exhibits anti-thrombotic properties, and may be utilized in catheters, tubes, sheets or in medical implements, such as oxygenators or pump-oxygenators employing them.

Bundesdruckerei Berlin

Description

Medical Material and Medical Implement

BACKGROUND OF THE INVENTION

This invention relates to a medical material exhibiting superior anti-thrombotic properties, and a medical instrument or implement making use of such material.

A variety of materials exhibiting anti-thrombotic properties have so far been devised for utilization in pump oxygenators, catheters or artificial hearts. As an example, a method for fixing or immobilizing heparin on the substrate surface has been devised. Thus there are known methods consisting in ionically bonding heparin to the substrate, and in covalently bonding heparin to the substrate.

However, with ionic bonding, surface activity is insufficient because of possible detachment of heparin on contact with blood or of too strong bonding with sulfuric group which is critical for demonstration of activities proper to heparin. With covalent bonding, surface activity proper to heparin is similarly insufficient because the bonding point of heparin with the substrate is the bonding point with AT-III. Although an attempt has been made to ionically bond heparin to the cationic surface and to cross-link heparin with glutaraldehyde, the desired effect is not sufficiently persistent because the aldehyde groups may react mainly only with primary amino groups, for example, so that there exist only few primary amino groups in heparin and covalent bonding with the substrate cannot occur in the absence of the primary amino groups in the substrate. While a method including coating a prepolymer is frequently adopted for heparin immobilization, this method has a drawback that polymer peeling or cracking may frequently occur in portions where resilient properties are a requirement.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to overcome the above problems and to provide a medical material having superior anti-thrombotic properties, and a medical implement utilizing such material as well as a method for manufacturing the same.

In view of the fact that, when it is simply tried to immobilize heparin to the substrate surface, sufficient anti-thrombotic properties cannot be produced, or effective positions are used up for this immobilization, the present inventors have conducted eager researches, and have won a success in immobilizing heparin without using the effective positions and in stably immobilizing heparin even to resilient substances to arrive at the present invention.

Thus there are utilized in heparin the N-sulfuric acid positions which are partially desulfated to produce primary amino groups.

According to the first aspect of the present invention, the substrate is conditioned so that it has a functional group, especially the primary amino group, on its surface. As an illustrative example, the method for preparing a substrate having a primary amino group on the surface is explained. Although this method may be selected optionally, a method including coating a compound having primary amino groups on the substrate, a method including coating a compound containing a primary amino group, coating a compound containing glycidyl or aldehyde groups and bonding a compound containing a primary amino group thereto, or a method including bonding a functional group on the substrate to a compound having a primary amino group and capable of reacting with the functional group on the substrate.

The substrate and heparin are conditioned in this manner and heparin is immobilized on the substrate surface. It is preferred for heparin to be immobilized to the compound having the primary amino group through the use of a compound having two or more aldehyde groups, such as glutaraldehyde.

It becomes possible in this manner to fix the portion of heparin unnecessary for anticoagulant activities proper to heparin selectively to the substrate.

According to the second aspect of the present invention, it is preferred to introduce the functional group capable of bonding with heparin either directly or through one or more coupling agents by way of an ozonating treatment of the substrate. This functional group is preferably a primary amino group, an aldehyde group or an epoxy group.

The substrate and heparin are conditioned in this manner and heparin is fixed to the substrate surface. The fixing of the compound having the above functional group on the substrate surface to heparin is performed in the form of covalent bond either directly or through one or more coupling agents. The compounds having at least two aldehyde groups, such as glutaraldehyde, may be employed as at least one of these coupling agents.

According to the third aspect of the present invention, the substrate is prepared in such a manner as to have a functional group, preferably a primary amino group, on its surface. Although the method for preparation may be arbitrary, it is preferred to introduce the functional group capable of bonding to heparin on the substrate through a compound such as is exemplified by hydroxyethyl methacrylate (HEMA) or methyl methacrylate (MMA). This functional group is preferably a primary amino group or an epoxy group.

Preferably, hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) exist as respective separate segments and are of such a structure in which the functional group exists in the hydroxyethyl methacrylate segment.

Preferably, the compound containing hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) has the contents of not less than 30% of hydroxyethyl methacrylate (MMA).

The substrate and heparin are prepared in this manner and heparin is fixed to the substrate surface. The fixing of the compound having the above functional group on the substrate surface with heparin is performed either directly or through one or more coupling agents in the form of covalent bonds. The compounds having at least two aldehyde groups, such as glutaraldehyde, may be employed as at least one of these coupling agents.

The medical materials obtained by the above described methods according to the first to third aspects of the present invention may be utilized as a variety of anti-thrombotic materials, which in turn may be used above all in the medical implements having portions contacting with blood. Examples of the medical materials include hollow fiber, tube or sheet, whilst examples of the medical implements include oxygenators, pump-oxygenating circuits, catheters or artificial hearts.

Porous membranes or films having numerous micropores are preferably employed as the hollow fibers and oxygenators, these micropores being preferably filled with fine particles of, for example, silica, having lesser particle size than the pore diameter.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a chart showing desulfation of N-sulfuric acid positions of heparin and changes in the anti-FXa and anti-FIIa activities proper to heparin.

Fig. 2 is a chart showing what value, as measured by the ninhydrin method, the amount of the primary amino group has by the desulfation of the N-sulfuric acid position of heparin.

Fig. 3 is a chart showing surface activities of heparin of a sample of a medical material obtained by Example 1-6.

Fig. 4 is a chart showing surface activities of heparin in samples of medical materials obtained by Example 2-4.

Fig. 5 is a chart showing surface heparin activities in samples of medical materials obtained in Example 3-5.

DETAILED DESCRIPTION OF THE INVENTION

For better understanding of the present invention, the relation between the different aspects of the present invention will be explained. In sum, the present invention includes three aspects I, II and III, including in turn separate respective Examples.

(A) First Aspect

A material in which the functional group is introduced on the substrate and coupled directly or through a coupling agent to heparin, application of the material to a medical implement, and the method for producing the material, which are described in claims 1, 4, 8 to 11, 12 and 16 to 24.

(B) Second Aspect

The material, application to the implement and the method similar to those of the first aspect mentioned above, with the exception that the functional group is introduced to the substrate with the medium of an oxide, which are described in claims 2, 5, 8 to 11, 13 and 17 to 24.

(C) Third Aspect

The material, application to the implement and the method similar to those of the first and second aspects mentioned above with the exception that the functional group is introduced to the substrate with the medium of a compound mainly containing HEMA and MMA, which are described in claims 3, 4, 6, 7, 14, 15 and 17 to 24.

(A) First of all, the first aspect of the present invention will be explained.

In the medical material of the present invention, heparin, preferably heparin having a portion of its N-sulfuric acid desulfated and thereby turned into primary amine, is fixed or immobilized to a substrate having a functional group, preferably a primary amino group.

First, the substrate including primary amine, a preferred functional group for fixing heparin, is explained.

As the substrate, polypropylene, polyvinyl chloride, polyurethane or glass, is generally employed, according to the usage and application.

In general, such substrate itself lacks in primary amine. In this case, the primary amine is introduced to the substrate. While there are a variety of methods therefor, the following methods are preferred.

The first method is coating a compound containing a primary amino group on the substrate surface. Examples of this type of the compound include chitosan or polyethylene imine (PEI).

The second method is coating a compound capable of reacting with the compound having the primary amino group and causing these compounds to react with and be bonded to each other. The compounds coated on the substrate surface include those having glycidyl groups, such as, for example, polyglycidyl methacrylate.

The compounds having the primary amino groups capable of reacting with the compounds coated on the substrate include ethylene diamine or polyethylene glycol diamine. At this time, two or more amino groups are required each for bonding both to substrate and heparin.

The third method is reacting a compound having both the primary amino group and the functional group

capable of reacting with the functional group on the substrate with the substrate and bonding it to the substrate. An example of this type of the compound is α-aminopropylethoxysilane when fixing heparin to a glass substrate.

Heparin to be fixed to the substrate having the primary amino group as mentioned above will be explained.

Heparin is well known as a compound exhibiting anti-thrombotic properties, and has a N-sulfuric acid portion in the form of $NHSO_3Na$. Heparin when fixed directly to the substrate surface raises problems, as mentioned above. It is therefore preferred in accordance with the present invention to desulfate and convert a portion of the N-sulfuric acid position into a primary amine. The degree of amination is preferably such that the primary amino groups account for not less than 5% and not more than 15% based on the total number of the amino groups present in heparin, since the amount of the primary amine lesser than the above lower limit lowers the amount of covalent bonds and the amount of the primary amino group more than the upper limit lowers the activity proper to heparin. The preparation of the partially desulfated heparin will be explained in the Examples.

Then, the fixing will be described between the substrate thus obtained including the primary amino group and heparin partially desulfated and primary aminated.

For fixing the substrate and heparin to each other, a compound having at least two aldehyde groups is used and the primary amines of both the substrate and heparin are bonded to each other by the reaction between the primary amino group and the aldehyde group. The aldehyde containing compound may be exemplified by glutaraldehyde.

The medical material in which heparin having its N-sulfuric acid partially desulfated to primary amine is fixed to the substrate having the primary amino group represents an anti-thrombotic material which makes use of the anti-thrombotic properties of heparin, and may be employed in a variety of medical implements, such as, for example, catheters and artificial hearts. Above all, when the porous membrane having numerous micropores for use as the gas exchange film or membrane , such as hollow fiber, is processed as described above, the hollow fiber exhibiting anti-thrombotic properties may be produced. On the other hand, when the porous membrane, such as the hollow fiber, is used in the oxygenator, an oxygenator having superior anti-thrombotic properties may be produced.

The micropores of the porous membrane employed in the oxygenator are preferably filled with fine particles lesser in diameter than the pores. It is because the gas exchange film is porous and hydrophobic and hence cannot be coated uniformly with a polymer, so that the anti-thrombotic properties cannot be exhibited fully, while, on the other hand, the membrane is hydrophilized due to fixing of heparin and hence the plasma tends to be exuded and leaked through the pores in case of prolonged circulation.

The fine particles may be packed in the porous membrane as described in the Japanese Patent Application KOKAI No. 64374/1987. This will be explained briefly hereinbelow.

A liquid dispersion of fine particles having the particle size lesser than the micropores of the porous membrane is caused to flow through the porous membrane so that the pores are stopped up with the particles.

As the materials for these fine particles, inorganic materials, such as silica, alumina, zirconia, magnesia, barium sulfate, calcium carbonate, silicate, titanium oxide, silicon carbide, carbon black or white carbon, or high polymer latices, such as polystyrene latex, styrene rubber (SBR) latex and nitrile rubber (NBR) latex, may be employed. Silica, above all, is the preferred material. The mean particle size of these fine particles is 0.003 to 1.0 μm and preferably 0.003 to 0.5 μm. These fine particles are in the form of a liquid dispersion and applied as such to the gas exchange membrane. A dispersion medium stable against the particles and the gas exchange membrane, such as water and alcohol, may be employed. However, when the dispersion medium is water, and the gas exchange medium is hydrophobic, it is necessary to hydrophilize the surface of the gas exchange membrane by contacting alcohols, such as ethanol and methanol with the surface of the gas exchange membrane prior to causing the liquid dispersion to flow therethrough.

When the gas exchange membrane is a hollow fiber, the liquid dispersion of the fine particles may be passed from the inside of the fiber under a moderate pressure to effect the packing of the fine particles more satisfactorily.

(B) The second aspect of the present invention will now be explained only with reference to the difference thereof from the first aspect.

In the medical material of the present invention, heparin having a portion of N-sulfate groups thereof desulfated and converted into primary amines is fixed either directly or through one or more coupling agents to a substrate having functional groups, such as primary amino group, epoxy group and aldehyde group.

The substrate having the above functional group is first explained.

As the substrate, polypropylene, polyurethane or polyvinyl chloride is generally employed, according to the usage and application.

In general, such substrate itself lacks in the functional group. In this case, the functional group is introduced to the substrate. While there are a variety of methods therefor, the following methods are preferred.

According to the present invention, ozone oxidation is utilized for introducing the above functional groups. In general, on oxidation of organic compounds with ozone, various functional groups, such as aldehyde, ketone and epoxy, are produced.

It has thus been found advisal an expedient to treat the substrate with ozone and to immobilize heparin through the use of these highly reactive functional groups. Although it is possible to fix heparin directly to

these functional groups, the problem of steric hindrance is raised. Thus the method consisting in introducing a spacer or a coupling agent into these functional groups and to fix heparin with the aid of this spacer is most useful because of ease of operation and exhibition of surface activity proper to heparin.

By the above technique, the functional groups can be introduced directly into a resilient material, such as polyurethane and polyamide/polyether copolymer, so that heparin can be bonded to the polymer easily without peeling on the polymer surface.

The explanation on heparin to be fixed to the substrate having the above described functional groups is made in the first aspect and the Examples 1-4.

For fixing the substrate and heparin to each other, a compound having at least two aldehyde groups is used as at least one of the coupling agents, and the primary amine is reacted with the aldehyde group. Such aldehyde compound may include glutaraldehyde. The coupling agent may be exemplified by the aldehyde compound and an epoxy compound, such as polyethylene glycidyl ether. In these cases, it is preferred for a coupling agent having two or more primary amino groups, such as polyethylene glycol diamine and polyethylene imine, to be previously coupled to the functional group obtained by an ozonating treatment.

The substrate and an heparin may be bonded directly to each other, in which case the epoxy group or the aldehyde group, that may be bonded to the amino group, represents the common connecting point, as the functional group.

The medical material in which heparin having its N-sulfuric acid partially desulfated to primary amine is fixed to the substrate having the above functional groups represents an anti-thrombotic material which makes use of the anti-thrombotic properties of heparin, and may be employed in a variety of medical implements, such as, for example, oxygenators, pump-oxygenating circuits, catheters or artificial hearts, including portions contacting with the blood. Above all, a pump-oxygenator or a self-retaining catheter formed of the above material is excellent in anti-thrombotic properties, while being stable against breakage, bending or clamping. On the other hand, when the porous membrane having numerous micropores for use as the gas exchange film or membrane, such as hollow fiber, is processed as described above, the hollow fiber exhibiting anti-thrombotic properties may be produced and, when the porous membrane, such as the hollow fiber, is used in the oxygenator, an oxygenator having superior anti-thrombotic properties may be produced.

The micropores of the porous membrane employed in the oxygenator are preferably packed with fine particles lesser in diameter than the pores. The reason is that, when the gas exchange film is porous and hydrophobic and also the functional group are not produced on ozonating treatment, a polymer producing the functional groups need be applied previously, in which case, however, the gas exchange film cannot be coated uniformly with a polymer, so that the anti-thrombotic properties cannot be exhibited fully, while, on the other hand, the membrane is hydrophilized due to fixing of heparin and hence the plasma tends to be exuded and leaked through the pores in case of prolonged circulation.

The packing of the fine particles in the porous film is as described in the first aspect of the present invention.

(C) The third aspect of the present invention will be explained only with reference to the difference from the first aspects thereof mentioned above.

In the medical material of the present invention, heparin having its N-sulfate groups partially desulfated to primary amine is fixed to the substrate having functional groups, such as amino groups, preferably primary amino groups.

The substrate having the above functional groups is first explained.

As the substrate, polypropylene, polyvinyl chloride or polyurethane is generally employed, according to the usage and application.

In general, such substrate itself lacks in primary amine. In this case, the primary amine is introduced to the substrate. While there are a variety of methods therefor, the following methods are preferred.

According to the present invention, a polymer including hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) is preferably employed as the material introducing the functional group to the substrate.

This polymer is preferred because the polymer itself has superior adaptability to blood and to living body and high safety, while it can be synthesized and coated relatively easily.

This polymer including HEMA and MMA components is preferably in the form of a block copolymer and the HEMA and MMA components form separate segments A and B, respectively. These segments are preferably separated from each other in the polymer structure. The reason is that, when the respective components are bonded to each other in separate segments, the segments containing MMA having higher water-proofness can be bonded intimately to the substrate. The segments A and B herein mean the fractions or portions containing HEMA and MMA, respectively.

The aforementioned functional groups are caused to exist in the segment A containing hydroxyethyl methacrylate (HEMA). These functional groups may include primary amino group and epoxy group.

For affording these functional groups to the HEMA segment, the compounds containing glycidyl methacrylate (GMA) may be contained in the HEMA segment besides HEMA. Preferably, the epoxy group in GMA and the compound having two or more primary amino groups are reacted with each other to introduce the primary amino groups on the surface.

Similarly, compounds other than MMA may also be contained in the segment B mainly containing MMA.

However it is preferred for the compound containing hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) to contain not less than 30% of hydroxyethyl methacrylate (HEMA). The reason is that, with the lesser amount of HEMA, the surface tends to become hydrophobic and the introduced functional

groups tend to appear on the surface only difficultly during the reaction in the aqueous solution.

Heparin to be fixed to the substrate having the aforementioned primary amino groups is explained in the first aspect and Examples 1-4.

The fixing of heparin to the substrate is as explained in the second aspect except for those portions relating with ozonating treatment. The application to medical implements, porous membranes used in oxygenators, and the packing of fine particles in the porous membranes are as already described in the first and second aspects.

EFFECT OF THE INVENTION

According to the present invention, predetermined functional groups are previously introduced onto the substrate, whilst N-sulfuric acid position of heparin is partially desulfated and converted into primary amino groups and the functional groups of heparin and the substrate are bonded to each other directly or with the intermediary of coupling agents for immobilizing heparin to the substrate. Thus the medical materials and, above all, the medical implements making use of these materials, such as the oxygenators, are markedly improved in anti-thrombotic and anti-plasma-leakage properties, with the result that the materials or implements can be used over an extended period of time.

EXAMPLES

The first aspect of the present invention will be explained with reference to several Examples.

I. Introduction of Amino Group on the Substrate

Example 1-1

Chitosan was dissolved in formic acid to prepare a 1% W/V solution. This solution was cast in a shallow vat formed of a teflon sheet and dried for two days at room temperature in an air draft. The dried material was peeled off carefully from the teflon sheet to produce a chitosan film. This film was immersed for three hours at room temperature in a 100 mM pH 10.0 carbonic acid buffer containing 1 % of polyethylene glycol having epoxy groups affixed to its both ends, marketed by Nagase Kasei Co., Ltd. under the trade mark of Denacol (molecular weight, 1,000) to cross-link chitosan partially to produce an unsolubilized chitosan membrane or film.

Example 1-2

PP microporous film, marketed under the trade mark of Duragard by the Polyplastic Co., Ltd was immersed for five minutes in 0.4% $KMnO_4$/conc H_2SO_4 to hydrophilize the surface with $-SO_3$. After washing with water thoroughly, the film was immersed at 45°C for two hours in a 0.1%-aqueous solution of PEI, pH 10, to immobilize polyethylene imine (PEI).

Example 1-3

γ-aminopropylethoxysilane was dissolved in ethanol to prepare a 1%-solution. A slide glass was washed carefully with a neutral detergent, dried, immersed in the above silane coupling agent and reacted for two hours in an oven maintained at 60°C to introduce NH_2.

II. Preparation of Partially N-desulfated Heparin

Example 1-4

Commercially available heparin was dissolved in distilled water to prepare a 10%-heparin solution. 0.4 ml of 5.5 N H_2SO_4 was added to 10 ml of this heparin solution and reacted at 95°C. The reaction mass was sampled periodically and the increase in the amount of the primary amino groups was measured by a ninhydrin method, whilst anti-FXa and anti-FIIa activities, among the heparin activities, were measured by the synthetic substrate method making use of S-2222 and S-2238. The results are shown in Fig.1.

A reaction was conducted under the condition that, a literature say, all the sulfoamino groups in heparin would be desulfated, and the reaction mass was sampled periodically and the increase in the amount of the primary amino groups was measured. The results are shown in Fig. 2.

It is seen from Fig. 2 that, when the sulfoamino groups are converted as the whole into primary amino groups, the amount of the primary amino groups of 0.9 μmol/10 mg is indicated by the ninhydrin method.

On the other hand, it is seen from Fig. 1 that the heparin activity is lowered with increase in the amount of primary amino groups and the excess desulfation of N-sulfuric acid of heparin is not preferred. For maintaining the heparin activity to some extent, it is preferred to maintain the primary amino groups in heparin at the level of 10 to 20% in -N sulfuric acid from the value of the amino group when converted into the whole sulfoamino group in Fig. 2 and from that of the amino group of Fig. 1. Hence, in the Examples to follow, aminated heparin obtained by the reaction continuing for 15 minutes is employed.

Meanwhile, the ninhydrin reagent is prepared by dissolving 2 g of ninhydrin and 0.3 g of hydrindantin in 75 ml of methyl-cellosolve and adding 25 ml of 4N sodium acetate (pH, 5.5).

To 0.75 ml of a sample to be tested is added 0.5 ml of the ninhydrin reagent and the resulting mixture is heated in boiling water for 15 minutes. The heated mixture was cooled rapidly and admixed with 5 ml of

25%-ethanol and the light absorbance was measured at 570 nm. The quantity of the amino groups was determined by the coloration degree of leucine.

In Fig. 1, the mark ○ indicates anticoagulancy against activated second blood clotting factor, whereas the mark ● indicates anticoagulancy against activated tenth blood clotting factor. There are a clotting factor for which heparin exhibits its anticoagulancy only in the high molecular weight range and a clotting factor for which heparin exhibits its anticoagulancy even in the low molecular weight range. In Fig. 1, activated second and activated tenth factors, representative of the clotting factors, are taken as examples to illustrate that heparin shows about the same anticoagulant activities for both the high and low molecular weight ranges.

It is seen from Figs. 1 and 2 that the amount of the primary amino groups in heparin is increased with incubation time, but the activities proper to heparin are lowered gradually, as shown in Fig. 1. It is therefore necessary to turn the N-sulfuric acid position of heparin into primary amine in the region in which the activities proper to heparin are not lowered inappropriately.

III. Fixing of Heparin to Substrate

Example 1-5

The substrates obtained in accordance with Examples 1-1 to 1-3 were immersed in a 0.5%-solution of partially aminated heparin (pH, 4.0; 100 mM of acetic acid buffer) at 45°C for two hours and allowed to stand overnight at room temperature in 2.5% glutaraldehyde acetic acid buffer (pH; 4.0). The substrates were then immersed in a 1% $NaBH_4$ carbonic acid buffer, pH 10.0, for three hours at room temperature and reduced to produce sample films on which heparin remains fixed (samples A, B and C).

The substrates produced in the Examples 1-1 to 1-3 were processed in the similar manner with heparin not aminated partially to produce sample films (samples D, E and F).

The samples A to F were dyed with 0.04% toluidine blue. It was found that the samples A to C were stained in red to purple color, whereas the samples D to F were scarcely stained.

Example 1-6

0.4%-$KMnO_4$/conc H_2SO_4 was maintained for two minutes on the inside surface of a polyethylene (PE) tube having the inside diameter of 1.4 mm and washed with water to hydrophilize PE tube. The inner surface of the hydrophilized PE tube was coated with chitosan of Example 1-1 and similarly insolubilized.

PEI was similarly immobilized to hydrophilized PE as in Example 1-2. Partially aminated heparin was immobilized to these two samples in accordance with Example 1-5 (samples G and H).

Antithrombotic surface activities of these two samples were measured with a synthetic substrate S-2238. In more detail, a tube on which heparin was fixed was cut to a length of 56 cm and 0.5 ml of 0 to 10 U/cc of thrombin (4% Alb saline solution) was introduced into the tube and allowed to contact with the inner surface of the tube for 15 minutes. The thrombin concentration of the inside liquid was measured to compute the amount of thrombin adsorbed to the inner tube surface. The tube to which thrombin was adsorbed was washed with a saline solution. Then, 1.0 ml of a detergent liquid (0.6 mM S-2238) was caused to flow through the inside of the tube at the rate of 2 ml/min and the liquid flowing out of the tube was caused to flow dropwise into 0.2 ml of 50% acetic acid to terminate the reaction. The light absorbancy of the reaction solution was measured and an analytical curve for color developing properties of S-2238 with respect to the amount of thrombin adsorbed to the inner surface was prepared.

Then, 1U/cc of AT-III was introduced into a tube to which 10U/cc of thrombin was adsorbed. After incubation, S-2238 was similarly subjected to color development with thrombin remaining on the inner surface and the amount of thrombin remaining on the inner surface was calculated from the degree of color development and the analytical curve.

The changes in the amount of thrombin remaining on the inner surface with the incubation time for AT-III being changed are shown in Fig.3. In the above, thrombin was inactivated by AT-III.

Measurement was not performed for the Example 1-3 since the measurement was difficult to perform because the substrate was formed by a glass.

IV. Preparation of Oxygenator

Example 1-7

Into an oxygenator of 0.8 m^2 formed of microporous PP hollow fiber (inside diameter, 200 μm, porosity of 50% and a mean pore diameter of 800 Å) was infused 0.1% chitosan / formic acid solution and air was blown via a blood port, while the gas port was aspirated by an aspirator, for coating chitosan on the inner surface of the oxygenator. Chitosan was insolubilized similarly to Example 1-1 and dried. This operation was repeated again for coating the chitosan film.

Example 1-8

0.1% of a PEI solution, pH 10.0, was infused into a microporous PP fiber oxygenator of 0.8 m^2 capacity and immersed at 45°C for two hours for turning the surface into PEI.

Example 1-9

600 ml of a liquid dispersion in water of silica having a mean diameter of 135 Å was passed through a hollow fiber employed in Example 1-7 for packing silica in the micropores of the hollow fiber.

This oxygenator was processed in the similar manner as in Example 1-7.

Example 1-10

The micropores of the oxygenator were packed with silica in the similar manner as in Example 1-9. The processing operation similar to that shown in Example 1-9 was then performed.

V. Fixing of Heparin to Oxygenator and Anti-thrombtic Properties

Example 1-11

Partially aminated heparin was immobilized to the oxygenator of Examples 1-7 to 1-10, in the similar manner as in Example 1-4.. The oxygenator thus treated were termed I, J, K and L, respectively.

A-V shunt of femoral artery and veins was performed of a mongrel weighing 20 kg, with each of the oxygenator, and anti-thrombotic properties were appraised. The comparative sample was a blank oxygenator.

This blank oxygenator was occluded in two hours, whereas the oxygenator I was occluded in four hours, the oxygenator J was occluded in five hours and the oxygenators K and L were not subjected to the lowering of the flow rate even after six hours.

VI. Measurement of Oxygenation and Decarbonation of Oxygenator

Example 1-12

The properties of decarbonation and oxygenation were measured of the oxygenators I to L. The results are shown in Tables 1-1 and 1-2.

Table 1-1

QB (l/min)	Blank	O_2 Transfer (V/Q = 1)(ml/min) I	J	K	L
0.3	21	19	20	20	21
0.5	32	27	32	28	30
0.8	26	40	45	41	42

Table 1-2

QB (l/min)	Blank	CO_2 Transfer (V/Q = 1) (ml/min) I	J	K	L
0.3	19	13	18	15	17
0.5	32	23	32	28	30
0.8	51	37	50	42	45

The results of the Examples 1-11 and 1-12 may be analyzed as follows:

In samples I and J, occlusion time is seen to be extended by coating. However, in sample I, chitosan coating becomes locally thick and the channel tends to become narrow or portions devoid of coating may be observed. The result is the lowered gas exchange properties. In sample J, occlusion takes place in five hours because of coating irregularities. The coating film thickness is so thin and a uniform film is not provided, so that the gas exchange properties are not lowered.

In samples K and L, in order to render the microporous film into a "hydrophilic supermicroporous film", the coating can be made uniformly and a thin and uniform film may be provided. Thus the sample K is simpler in the coating method than the sample I and has the improved gas exchange properties. In sample L, the coating becomes more uniform, so that occlusion does not take place even in six hours.

The second aspect of the present invention will be explained with reference to several Examples.

I. Introduction of Functional Groups to the Substrate

Example 2-1

Sheets of PEBA 6333SAOO and 2533SAOO, both a block copolymer of nylon and polyether, produced by Atochem Inc. and NKY-9LH, a solvent-soluble polyurethane, were prepared and termed A, B and C, respectively. The sheet A was processed by an ozonator produced by Nippon Ozone Co., Ltd. under

conditions of 0.8 lit/min of O_2 and 50°C for 10 minutes, while the sheets B and C were processed for 20 minutes under otherwise the same conditions. These processed sheets were termed A1, B1 and C1, respectively.

The changes in the ratio of the surface element composition were measured by ESCA before and after the ozonating treatment. The results are shown in Table 2-1.

The aldehyde on the suface was ascertained using a Shiff's reagent. It was found that the samples subjected to ozonating treatment A1, B1 and C1 were stained in a red purple to blue purple tint, whereas those not subjected to ozonating treatment A, B and C were not stained.

Table 2-1

Analysis of Surface Composition by ESCA

	A	A1	B	B1	C	C1
O	17.12	29.16	21.25	25.85	18.39	22.43
N	0.80	1.43.	1.70	4.78	3.36	4.17
C	82.05	69.74	77.04	69.36	78.25	73.40

II. Preparation of Partially N-Desulfated Heparin

Example 2-2

The commercially available heparin was dissolved in distilled water to prepare a 10%-heparin solution. 10 ml of this heparin solution were charged into 0.4 ml of 5.5N sulfuric acid and incubated at 97°C for ten minutes.

The amount of the primary amino groups in the produced heparin was 11% of total amino groups in untreated heparin. 11% were inclusive of those possessed by heparin from the outset and those produced by desulfation of sulfuamino groups.

III. Heparin Fixation to Substrate and Evaluation

Example 2-3

The sheets A, B, C, A1, B1 and C1, produced in accordance with the Example 2-1 were immersed at 45°C for 24 hours in 1.7% PGD-10 (polyethyleneglycol diamine) or 0.5% polyethylene imine (PEI), manufactured by BASF, adjusted to pH of 10. Then, acetic acid buffer solutions, pH 4.5, were prepared of 0.5% partially desulfated heparin reacted according to Example 2-2 and heparin prior to the reaction and the films were immersed in these solutions at 45°C for 24 hours. The films were then immersed in a 2.5% glutar aldehyde acetic acid buffer solution, pH 4.5, at room temperature for 24 hours and then in 1% $NaBH_4$ carbonic acid buffer solution, pH 10, at room temperature for four hours.

The sheets thus processed were immersed in 0.01N hydrochloric acid and stained in toluidin blue. It was found that the sheets A, B and C not subjected to ozonating treatment were scarcely stained.

The sheets subjected to ozone treatment A1, B1 and C1 were stained only slightly when reacted with heparin prior to reaction, while the same sheets when reacted with partially desulfated heparin were stained in purple to red purple color.

The sheets A, B, C, A1, B1 and C1 produced in accordance with Example 2-1 and processed similarly except the process of reacting with PEI and PGD-10 were stained in blue purple color when they were reacted with partially desulfated heparin after ozonating treatment.

Example 2-4 Anti-Thrombotic Properties of Tube on which Heparin was Fixed

A tube having an inside diameter of 1.4 mm was prepared from a polymer B shown in Example 2-1. Also a polyurethane tube having an inside diameter of 1.4 mm and coated on its inner surface with polymer C was prepared. The inner sides of these tubes were ozonated under the same ozonating conditions as those of Example 2-1. Partially desulfated heparin obtained in accordance with Example 2-2 was fixed on these inner sides in accordance with Example 2-3. These samples were termed B2, C2, B3 and C3. The samples without ozonating treatment of the tube and subjected to heparin fixing treatment were also prepared and termed B4, C4, B5 and C5.

In the samples B2, C2, B4 and B4, and samples B3, C3, B5 and C5, polyethylene glycol diamine and polyethylene imine were used respectively as the coupling agents between the substrate and heparin.

These tubes were rinsed with a borate buffer solution, pH 9, for 15 hours and then rinsed with a phosphate buffer solution, pH 7.4, for two hours. Anti-thrombinic surface properties of each tube were then measured.

In more detail, a tube on which heparin was fixed was cut to a length of 56 cm and 0.5 ml of 0 to 10 U/cc of thrombin (4% Alb saline solution) was introduced into the tube and allowed to contact with the inner surface of the tube by use of a rotary mixer for 15 minutes. The thrombinic concentration of the inside liquid was measured to compute the amount of thrombin adsorbed to the inner tube surface. The tubes to which thrombin was adsorbed was washed with a saline solution. Then, 1.0 ml of a detergent liquid (0.6 mM S-2238) was caused to flow through the inside of the tube at the rate of 2 ml/min and the liquid flowing out of the tube

was caused to flow dropwise into 0.2 ml of 50% acetic acid to terminate the reaction. The light absorbancy of the reaction solution was measured and a calibration curve for color developing properties of S-2238 with respect to the amount of thrombin adsorbed to the inner surface was prepared.

Then, 1U/cc of AT-III was introduced into a tube to which 10U/cc of thrombin was adsorbed. After incubation, S-2238 was similarly subjected to color development with thrombin remaining on the inner surface and the amount of thrombin remaining on the inner surface was calculated from the degree of color development and the calibration curve.

The changes in the amount of thrombin remaining on the inner surface with the incubation time for AT-III being changed are shown in Fig. 4. It was found that the samples subjected to ozonating treatment exhibited thrombin activities.

The tube was further rinsed with plasma for 30 minutes and heparin washed out into plasma was washed. The Chandler's loop test was then conducted.

The results are shown in Table 2-2. It is seen from this table that the effect of ozonating treatment and heparin immobolization manifests itself in the actual blood.

The method and the significance of the Chandeller's loop test are explained hereinbelow.

By measurement of the time during which the fresh blood not treated for anticoagulancy becomes coagulated, it is possible to evaluate the level of anti-thrombotic properties of the material contacted by the blood.

This tube was cut to a length of 20 cm and 88 µl of fresh blood sampled from the veins of the rabbit's ears was intorduced into the tube segment which was bent into a self-closed loop and turned at 8 rpm.

The time until the blood is coagulated and start to turn with the tube was measured.

From the above results, it is seen that heparin is immobilized by ozone processing and anti-thrombotic propeties are afforded to the samples.

Table 2-2

Chandler's Loop Test (Total Blood Coagulation Time)

	B2	B3	C2	C3	B4	B5	C4	C5
RUN 1	40'15"	60' <			10'20"	12'40"		
RUN 2			27'25"	60' <			11'55"	12'20"
RUN 3	60' <	60' <			12'10"	13'45"		
RUN 4			26'30"	30'35"			11'20"	10'05"
RUN 5	52'50"	60' <	31'40"	60' <				
RUN 6					9'40"	10'15"	10'30"	11'10"

Example 2-5

Using a polymer B, the catheter with an inner diameter of 1.4 mm, an outer diameter of 2.2 mm and a length of 500 mm was produced. A polymer C was coated on the inner and outer surfaces of polyurethane to produce similarly the catheter with an inner diameter of 1.4 mm, an outer diameter of 2.2 mm and a length of 500 mm. The inner and outer surfaces of this catheter were treated with ozone and heparin was fixed to the inner and outer surfaces of the catheter similarly to the samples B3 and C3 of Example 2-4 to produce the catheter with heparin fixed thereto (samples B6 and C6). On the other hand, heparin was immobilized to the inner and outer surfaces of the catheter, similarly to the samples B4, C4, B5 and C5, without ozonating treatment, to produce catheters (B7, C7, B8 and C8).

With cannulation being made to left and right femoral veins of a mongrel weighting 15 kg and lactate ringel solution was caused to flow in the inside at a rate of 5 ml/hr, the catheter was left for 8 hours and then taken out to observe visually the degree of formation of thrombi in the catheter.

Thrombi were scarecely formed on the inner and outer sides of the catheters B6 and C6, whereas the inner sides of the Catheters B7, C7, B8 and C8 were substantially occulated while a large number of thrombi were affixed to the outer sides, thus demonstrating the favorable result of heparin immobilization by ozonating treatment.

Example 2-6

A polyether polyamide block copolymer was coated on the polypropylene hollow fiber type oxygenator (membrane surface, 0.8 m^2) having an inside diameter of 200 μm, membrane wall thickness of 25 μm, porosity of 45% and mean pore diameter of 700Å. The inner surface of oxygenator was then treated with ozone as in Example 2-1 and immersed in 0.5% PEI (polyethylene imine) adjusted to pH of 10 at 45°C for 24 hours.

Then, 0.5% acetic acid buffer solution of partially desulfated heparin reacted in accordance with Example 2-2 was prepared and the inner surface of oxygenator was immersed in this solution at 45°C for 24 hours. The inner surface of oxygenator was then immersed at room temperature for four hours in a 1% $NaBH_4$ carbonic acid buffer solution to produce an oxygenator A.

The gas exchange membrane of a similar oxygenator was hydrophilized by causing ethanol to flow into the oxygenator via blood inlet. After substitution with distilled water, the dispersion of colloidal silica having the mean particle size of 0.0125 μm in water was caused to flow into the oxygenator and filtrated through the gas exchange membrane to charge silica in the pores. Distilled water was then caused to flow to exclude the silica/water liquid dispersion remaining in the inside of the gas exchange membrane thoroughly before drying. The processing similar to that for the oxygenator A was performed to produce the oxygenator B.

A similar comparative oxygenator not treated with ozone was termed as the oxygenator C.

[Experiments for Evaluating Anti-thrombotic Properties]

A-V shunt of femoral veins and artery of a mongrel weighing 25 kg was performed with oxygenators A, B and C to cause the blood to be circulated at the maximum rate of 400ml/min.

It was found that no lowering of the flow rate was caused by the increased pressure loss during circulation continuing for eight hours for the oxygenator A or B.

The lowering of the flow rate was noticed for the oxygenator A after lapse of nine hours, but no such lowering of the flow rate was observed even after 12 hours for the oxygenator B.

Conversely, in the oxygenator C, circulation was rendered unfeasible in two hours due to formation of thrombi in the oxygenator.

Plasma leakage was observed after six hours in the oxygenator A, whereas no plasma leakage was seen to occur even after 12 hours in the oxygenator B.

Example 2-7

Polyurethane soluble in a solvent (NKY-9LH produced by Nippon Polyurethane Co., Ltd.) was coated on a soft vinyl chloride tube (CBT-650 produced by Terumo Kabushiki Kaisha). This tube was treated by an ozone generator for ten minutes at a flow rate of 0.8 lit/min of O_2 and at 50°C. This tube was immersed at 45°C for 20 hours in 0.05% polyethylene imine (PEI) and then at 45°C for four hours in 0.5% heparin (pH 4.0, 0.1 M acetic acid buffer).

On the other hand, polyepoxy compound (Denacol EX-421 produced by Nagase Co., Ltd.) and a diepoxy compound (Denacol EX-313 produced by Nagase Kasei Co., Ltd.) were dissolved in 10 mM of an acetic acid buffer, pH 4.0 to produce an aqueous solution of 5% polyepoxy compound and 10% diepoxy compound. Water-insoluble components were precipitated by centrifugation at 3000 rpm for ten minutes. The above tube was immersed in the recovered supernatant liquids as tubes I and II. 10 mM of acetic acid buffer solution not containing epoxy compounds, pH 4.0, was immersed in the tubes processed as above as tube III.

These tubes were reacted at 45°C for 20 hours and 44 hours, each tube showing as I-20 (hr), I-44(hr), II-20 (hr) and II-44 (hr). The tubes were then immersed for 16 hours in 1M ethanolamine, pH 10, before washing with water. These samples were stained with toluidine blue and the results as shown in Table 2-3 were obtained.

Table 2-3

hr	Ex. I	Ex. II	Comp. Ex. III
20	+	+	-
44	+++	+++	-

Example 2-8

A sheet of urethane soluble in a solvent (NKY-9LH) was prepared by dipping. This sheet was processed as in Example 2-7 and heparin was immobilized on this sheet. The sheet was then immersed in a polyepoxy and diepoxy compounds same as in Example 2-7 for 20 hours or 44 hours to perform an epoxy treatment (i), followed by ethanol amine treatment (ii) including immersion in 1M ethanol amine solution, pH 10.0, and further immersion treatment (iii) in 1M sodium chloride, pH 10, to remove ion-bonding heparin.

The ratio of the elementary composition of each sheet was measured at each stage of (i) to (iii). The results are shown in Table 2-4.

Table 2-4

(S element composition ratio in %)

	Ex. 1		Ex. 2		Cmp. Ex. III	
hr	20	44	20	44	20	44
(i)	1.91	2.07	2.03	1.96	2.03	2.03
(ii)	0.99	1.12	0.92	1.24	0.57	0.51
(iii)	0.93	1.17	0.94	1.28	0.60	0.53

Example 2-9

Polyurethane soluble in a solvent (NKY-9LH) was coated on the inner surface of a soft polyvinyl chloride tube (inside diameter, 1.4 mm) which was then treated in the same way as in Example 2-7. Heparinated tubes were produced in this manner for each of I-44 (hr), II-20 (hr) and II-4 (hr). Evaluation of anti-thrombotic properties was made of each tube by the Chandeller loop method.

For comparison and reference, the sample III in Example 2-7 was employed.

The evaluation was made on these samples directly and after incubation with 6% albumin for 24 hours three times and washing desorbed excess heparin thoroughly. The results are showin in Table 2-5.

It is seen from the above results that anti-thrombotic properties have been realized by fixing heparin to the epoxy compounds. It is felt that excess heparin has been substantially removed by processing with 1M ethanol amine processing at pH 10.

Table 2-5

	Evaluated Directly				Evaluated After Incubation with 6%-Albumin			
	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
I-44	60′ <	60′ <	60′ <	60′ <	60′ <	60′ <	60′ <	60′ <
II-20	25′45″	32′20″	27′10″	40′55″	20′25″	23′15″	19′55″	30′45″
II-44	60′ <	60′ <	60′ <	60′ <	60′ <	60′ <	60′ <	60′ <
III	10′35″	12′05″	8′50″	7′20″	8′30″	9′15″	5′50″	13′30″

Example 2-10

Polyurethane soluble in a solvent (NKY-9LH) was applied to the inner and outer surfaces of a soft vinyl chloride tube (inside diameter, 1.4 mm; outside diameter,2 mm) and heparinated tube was produced in the same way as in Example 2-7 in accordance with I-44 (epoxy compound 0% was processed as Comparative Example III for 44 hours).

With cannulation into left and right femoral veins and artery of a mongrel weighing 15 kg, and causing the inner side to flow with a lactate ringel at the rate of 5 ml/hr, the tube was left for 8 hours and then taken out to observe the degree of formation of thrombi visually. The sample I-44 was substantially free from thrombi, whilst in III numerous thrombi were noticed on both the inner and outer sides.

The third aspect of the present invention will be explained with reference to several Examples.

I. Introduction of Functional Groups to Substrate

Example 3-1

Polymers P1 to P4 of various compositions shown in the following Table 3-1 were prepared and coated on flat microporous polypropylene films. The polymer solutions were adjusted by diluting a 15% polymer solution in methyl cellosolve with methanol or a 9 : 1 methanol : acetone solution into a 2.5% polymer solution.

It is noted that epoxy groups are introduced into the segment containing hydroxyethyl methacrylate (HEMA) by bonding glycidyl methacrylate (GMA) whilst acrylic acid (AA) is introduced into the segment containing methyl methacrylate (MMA).

Table 3-1

Polymer Composition

	HEMA segment HEMA/GMA (wt.%)	MMA segment MMA/AA (wt.%)
P1	80/0	20/0
P2	68/12	17/3
P3	55/25	17/3
P4	40/40	17/3

II Preparation of Partially N-Desulfated Heparin

Example 3-2

The commercially available heparin was dissolved in distilled water to prepare a 10%-heparin solution. 10 ml of this heparin solution was charged into 0.4 ml of 5.5N sulfuric acid and incubated at 97°C for ten minutes.

The primary amino groups in the total amino groups in the produced heparin accounted for 11% of total amino groups in untreated heparin. 11% were inclusive of those possessed by heparin from the outset and those corresponding to the N-sulfuric acid positions desulfated and turned into primary amines.

III. Fixation of Heparin to Substrate and Evaluation

Example 3-3

A film prepared in accordance with Example 3-1 was immersed in 0.1% ethylene diamine and 1.7% PGD-10 (polyethylene glycoldiamine), adjusted to pH of 10, at 45°C and for 24 hours. Then, acetic acid buffer solution of pH 4.5 was prepared for each of 0.5% partially N-desulfated heparin reacted in accordance with Example 3-2 and heparin prior to reaction and the film was immersed in this solution at 45°C for 24 hours. The film was immersed in a 2.5% glutar aldehyde acetic acid buffer solution of pH 4.5 at room temperature for 24 hours and then in a 1% $NaBH_4$ carbonic acid buffer solution, pH 10, at room temperature for four hours.

These processed films were immersed in 0.01N hydrochloric acid and stained with toluidin blue. The films immersed in heparin not desulfated were substantially not stained, whereas the films immersed in partially desulfated heparin of polymers P2 to P4 were stained in red purple color. However, the polymer P1 was not stained. The films were also immersed in a borate buffer solution of pH 9 for 15 hours to remove ion-bonded heparin and dyed in a similar manner. It was found that there was no change except that the heparin immersed in film could not be stained.

Example 3-4 Ion Bonding Properties between Heparin and Substrate in Neutral Region

The film prepared in accordance with Example 3-1 was immersed in 0.1% ethylene diamine and 1.7% PGD-10 (polyethylene glycol diamine), adjusted to pH of 10, at 45°C for 24 hours. A 0.5% acetic acid buffer solution of pH 4.5 was prepared for each of partially desulfated heparin racted in accordance with Example 3-2

and heparin prior to reaction, and the film was immersed in this solution at 45°C for 24 hours. The film was then immersed in a phosphate buffer solution of pH 7.4 at room temperature for 24 hours and stained in the similar manner and was found to be scarcely stained.

From the fact stated, it follows that heparin and the substrate are not bonded ionically in the neutral range.

Example 3-5 Anti-Thrombotic Properties of Heparinized Tube

The polymer shown in Example 3-1 was coated on polyamide tubes each having the inside diameter of 1.4 mm and partially desulfated heparin produced in Example 3-2 similarly to Example 3-3 was fixed to the polymer. These tubes were washed in a borate buffer solution of pH 9 for 15 hours and then in a phosphate buffer solution of pH 7.4 for two hours and anti-thrombotic surface properties of each tube were measured. In more detail, a tube on which heparin was fixed was cut to a length of 56 cm and 0.5 ml of 0 to 10 U/cc of thrombin (4% Alb saline solution) was introduced into the tube and allowed to contact with the inner surface of the tube by use of a rotary mixer for 15 minutes. The thrombinic concentration of the inside liquid was measured to compute the amount of thrombin adsorbed to the inner tube surface. The tube to which thrombin was adsorbed was washed with a saline solution. Then, 1.0 ml of a detergent liquid (0.6 mM S-2238) was caused to flow through the inside of the tube at the rate of 2 ml/min and the liquid flowing out of the tube was caused to flow dropwise into 0.2 ml of 50% acetic acid to terminate the reaction. The light absorbancy of the reaction solution was measured and an analytical curve for color developing properties of S-2238 with respect to the amount of thrombin adsorbed to the inner surface was prepared.

Then, 1U/cc of AT-III was introduced into a tube to which 10U/cc of thrombin was adsorbed. After incubation, S-2238 was similarly subjected to color development with thrombin remaining on the inner surface and the amount of thrombin remaining on the inner surface was calculated from the degree of color development and the analytical curve.

The changes in the amount of thrombin remaining on the inner surface with the incubation time for ATIII being changed are shown in Fig.5. It was found that the samples stained to red to purple color exhibited activities.

It is seen from Fig. 5 that the polymer to which glycidyl group was introduced exhibited surface heparin activities and, above all, the polymer having higher HEMA contents exhibit such activities.

IV. Fixation of Heparin to Oxygenator and Evaluation

Example 3-6

A polypropylene hollow fiber type oxygenator having the inside diameter of 200 μm, wall thickness of 25 μm, porosity of 4% and a mean pore diameter of 700 Å (film surface, 0.8 m^2) was coated with the polymer P2 shown in Table 5. This oxygenator was immersed in 1.7% PGD-10 (polyethylene glycol diamine), adjusted to pH 10, at 45°C for 24 hours.

An acetic acid buffer solution, pH 4.5, of 0.5% partially N-desulfated heparin reacted in accordance with Example 3-2 was prepared and the oxygenator was immersed in this solution at 45°C for 24 hours. The oxygenator was immersed in an acetic acid buffer solution of pH 4.5 with 2.5% of glutar aldehyde at room temperature for 24 hours. The oxygenator was then immersed in 1% $NaBH_4$, pH 10 carbonic acid buffer solution at room temperature for four hours to produce an oxygenator A.

On the other hand, ethanol was caused to flow into the similar oxygenator via blood inlet to hydrophilize the gas exchange film. After substitution with distilled water, a dispersion of colloidal silica of mean particle size of 0.0125 μm in water was caused to flow into the oxygenator and filtrated through the gas exchange film for packing the silica in the pores. The distilled water was then introduced for dispalcing the dispersion in water of silica remaining in the inside of the gas exchange film and the oxygenator was then dried. The oxygenator was processed in the similar manner as for the oxygenator to produce the oxygenator B.

An oxygenator not coated with the polymer shown in Table 3-1 was prepared for comparison and reference as an oxygenator C.

Example 3-7 Evaluation of Anti-Thrombotic Properties

An A-V shunt was performed for femoral veins and artery of a mongrel weighing 25 kg, with respect to oxygenators A, B and C on which heparin was immobilized in accordance with Example 3-6 and the blood was circulated at the maximum rate of 400 ml/min.

There was no lowering of the flow rate caused by the increased pressure loss during circulation for eight hours through the oxygenators A and B.

The flow rate was seen to be lowered after 9 hours in the oxygenator A, whereas there was no lowering of the flow rate even after 12 hours in the oxygenator B.

Conversely, circulation was rendered unfeasible in two hours in the oxygenator C due to formation of thrombi in the oxygenator C. Plasma leakage was seen to occur after 6 hours in the oxygenator A, whereas such plasma leakage was not noticed even after 12 hours in the oxygenator B.

Claims

(1) A material for medical use comprising a substrate and heparin immobilized thereon, wherein the improvement resides in that
a functional group introduced on said substrate and a primary amino group of heparin are covalently bonded directly or through a coupling agent.

(2) The material according to claim 1 wherein said functional group is contained in an oxide on the surface of said substrate.

(3) The material according to claim 1 wherein said functional group is introduced on said substrate through a compound containing hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA).

(4) The material according to claim 1 or 3 wherein said functional group is a primary amino group.

(5) The material according to claim 1 wherein said functional group is aldehyde or epoxy group.

(6) The material according to claim 3 wherein said hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) exist in respective separate segments and wherein said functional group exists in the segment consisting of hydroxyethyl methacrylate (HEMA).

(7) The material according to claim 3 wherein said compound containing hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) contains not less than 30% of hydroxyethyl methacrylate (HEMA).

(8) The material according to any one of claims 1 to 7 wherein part of N-sulfuric acid of heparin is desulfated to primary amine.

(9) The material according to any one of claims 1 to 7 wherein the material is used as an anti-thrombotic material.

(10) A medical implement wherein at least a portion thereof contacting with the blood is formed of the material for medical use as claimed in any one of claims 1 to 9.

(11) A hollow fiber wherein at least a portion thereof contacting with the blood is formed of the material for medical use as claimed in any one of claims 1 to 9.

(12) A method for producing a material for medical use comprising the steps of
(a) introducing functional groups on a substrate, and
(b) covalently bonding said functional group and a primary amino group of heparin to each other directly or through a coupling agent.

(13) The method according to claim 12 wherein the step (a) of introducing said functional group on said substrate is performed by ozonating treatment.

(14) The method according to claim 12 wherein said step (a) of introducing said functional group on said substrate is performed through a compound containing hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA).

(15) The method according to claim 14 wherein said step (a) is performed by coating said substrate with a compound containing hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) and including an epoxy group, and thereafter reacting a compound having two or more primary amino groups therewith.

(16) The method according to claim 12 wherein said step (a) is performed by chemically bonding or coating a compound containing a primary amino group on said substrate.

(17) The method according to any one of claims 12 to 16 wherein said step (b) is performed by covalently bonding heparin having a portion of N-sulfuric acid thereof desulfated to primary amine to said functional group directly or through a coupling agent.

(18) The method according to any one of claims 12 to 17 wherein said coupling agent is a compound having at least two aldehyde groups.

(19) The method according to claim 18 wherein said compound having at least two aldehyde groups is glutaraldehyde.

(20) A method for producing a medical implement comprising the step of subjecting a contact surface of a substrate constituting the medical implement with the blood to the steps (a) and (b) as claimed in any one of claims 12 to 19 for affording anti-thrombotic properties to said contact surface.

(21) An oxygenator employing a porous membrane having a multiplicity of micropores as a gas exchange membrane, wherein a surface on which the blood is circulated is formed of the material for medical use as claimed in any one of claims 1 to 9.

(22) The oxygenator according to claim 21 wherein a multiplicity of fine particles having a particle size lesser than the diameter of the pore is packed in said micropores of said porous membrane.

(23) The oxygenator according to claim 22 wherein said fine particles are silica particles.

(24) A method for producing an oxygenator comprising performing said steps (a) and (b) as claimed in any of the claims 12 to 19 with the use as said substrate of a surface of a porous membrane on which the blood is circulated, said porous film being used as a gas exchange membrane and which is provided with a multiplicity of micropores.

F I G. 1

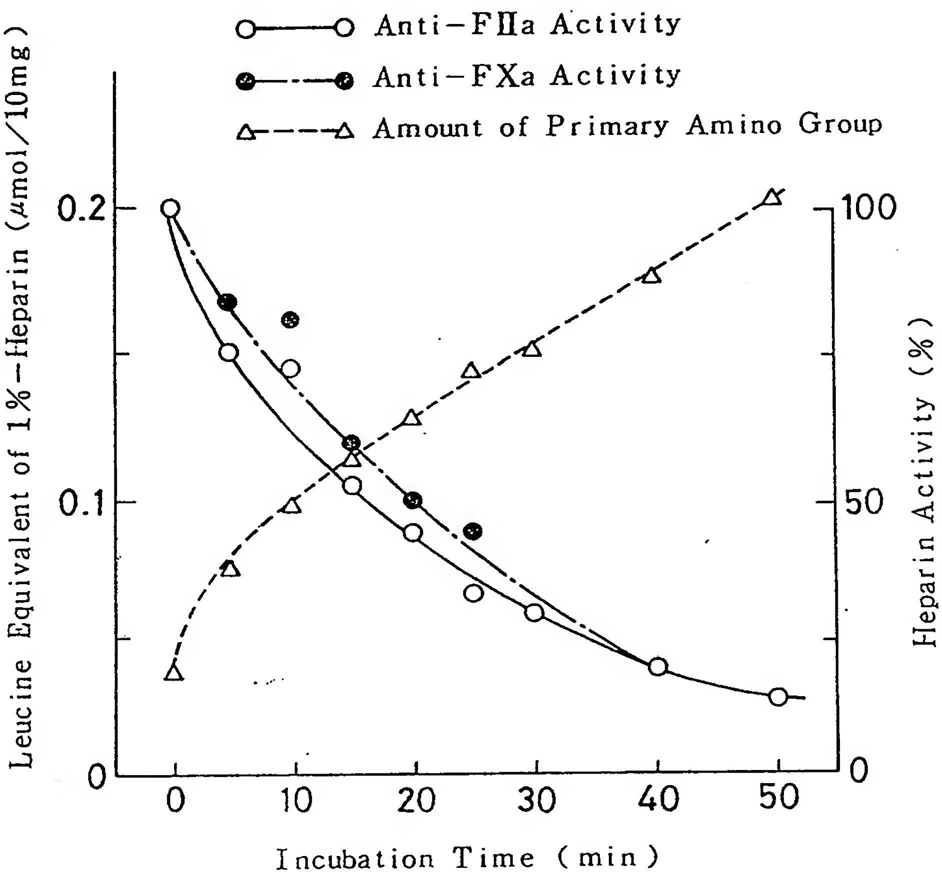

Anti−FIIa Activity
Anti−FXa Activity
Amount of Primary Amino Group
Leucine Equivalent of 1%−Heparin (μmol/10mg)
0.2
0.1
0
100
50
0
Heparin Activity (%)
0
10
20
30
40
50
Incubation Time (min)

FIG. 2

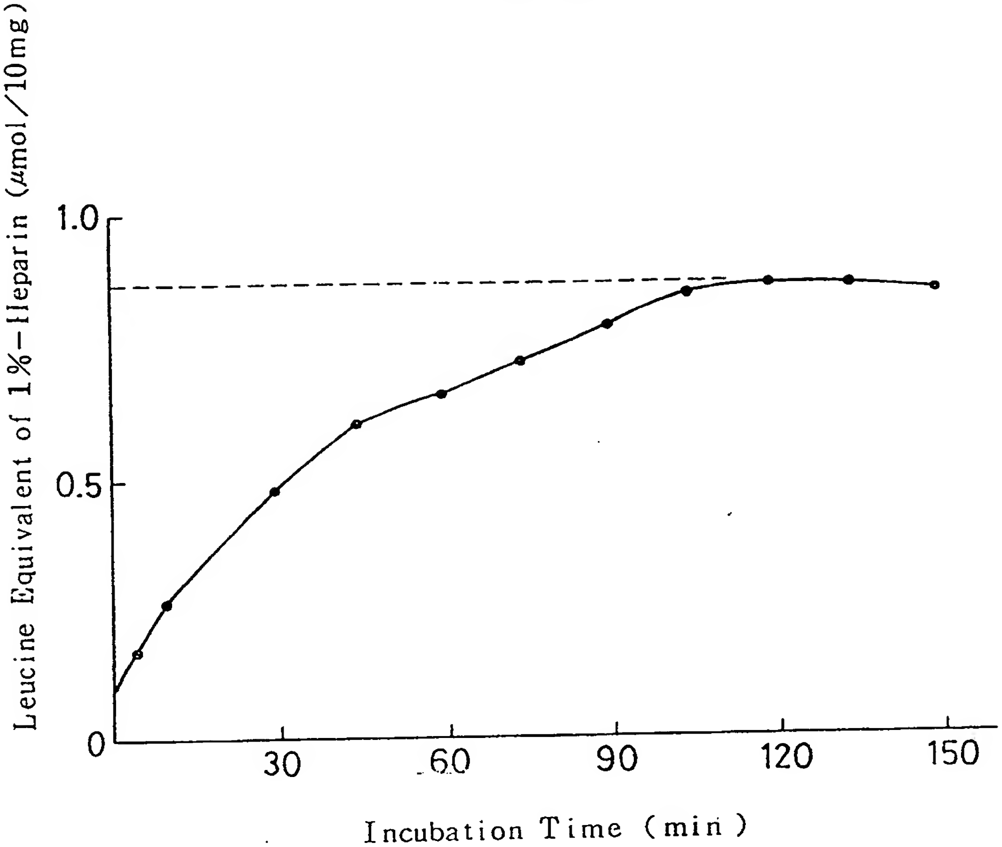
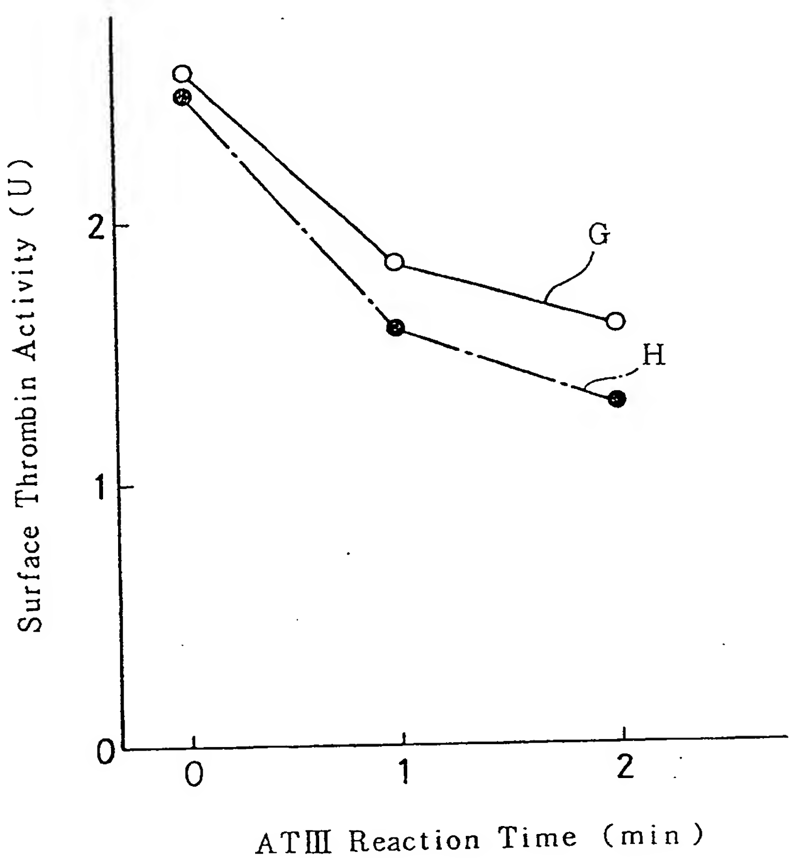
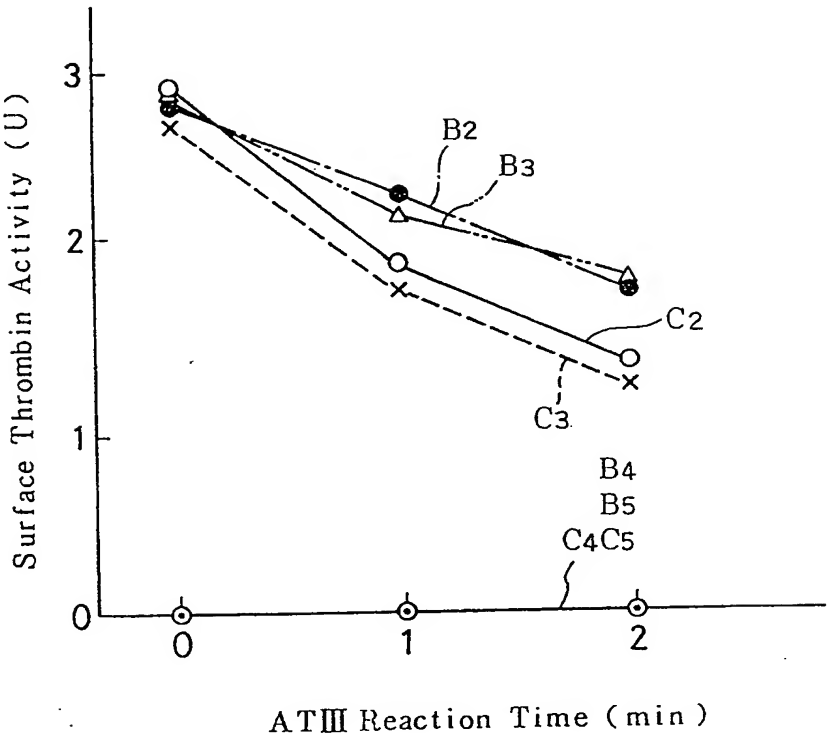

Leucine Equivalent of 1%-Heparin (μmol/10mg)
1.0
0.5
0
30
60
90
120
150
Incubation Time (min)

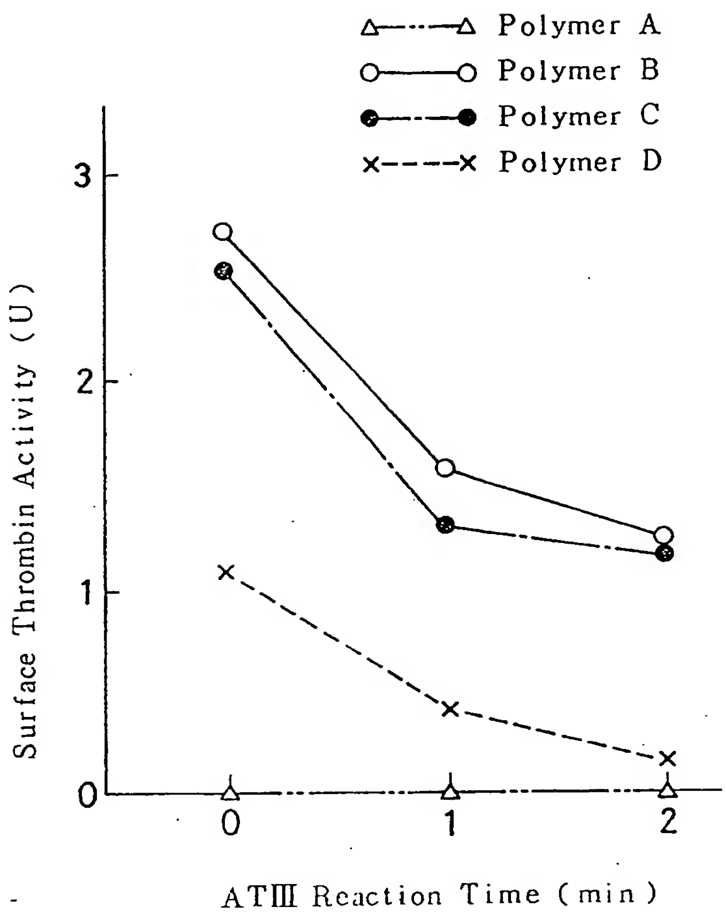
FIG. 3


Surface Thrombin Activity (U)
2
1
0
0
1
2
G
H
ATIII Reaction Time (min)

F I G. 4


3
2
1
0
Surface Thrombin Activity (U)
B2
B3
C2
C3
B4
B5
C4C5
0
1
2
ATIII Reaction Time (min)

F I G.5


Polymer A
Polymer B
Polymer C
Polymer D
3
2
1
0
Surface Thrombin Activity (U)
0
1
2
ATIII Reaction Time (min)